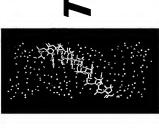
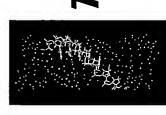
VIRAL RNA TARGET ENRICHMENT STRATEGY

By way of:



Triplex Formation



Triplex Formation

▼TARGET: POLYPYRIMIDINE STRAND

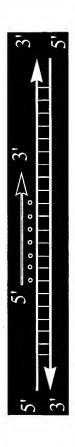
>PARALLEL-STRANDED HAIRPINS (HAIRPIN PROBES)

▼CONTAINING 8-AMINOPURINES

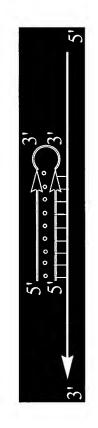
ABLE.

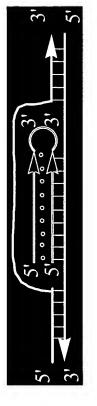
HIGHLY STABLE TRIPLEX STRUCTURES

PARALLEL-STRANDED HAIRPINS TRIPLE HELICES FORMED by



TARGET: POLYPYRIMIDINE STRAND







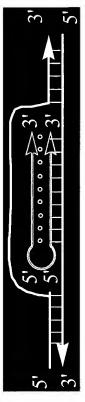


Figure 3

SYNTHESIS OF PARALLEL HAIRPINS

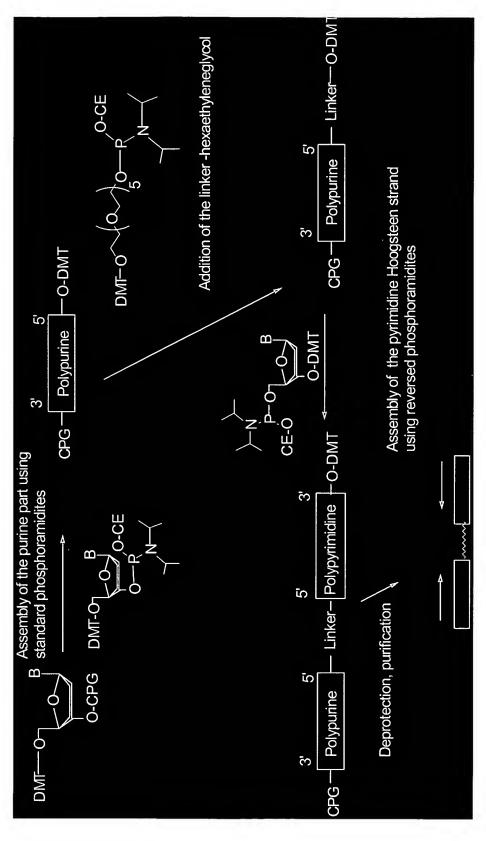


Figure 4

Synthesis of parallel-hairpins using asymmetric branching units

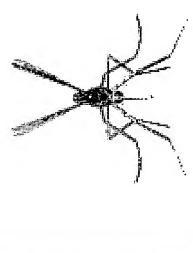
NHCO OTTAGAGGAGGAG-5' (SEQ ID NO: 23)

HOTO OTTTCTCCTCCTTC-5' (SEQ ID NO: 24)

∕o ³ù u u c u c c u c c u u c⁵ \sim O 3 † TA G^NAGG^NAG G A A G⁵′ (SEQ ID NO: 20) (SEQ ID NO: 21) AR22A-RNA

Figure 5

WNV INFECTED SAMPLES



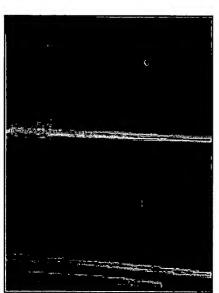


Figure 6

Strategy

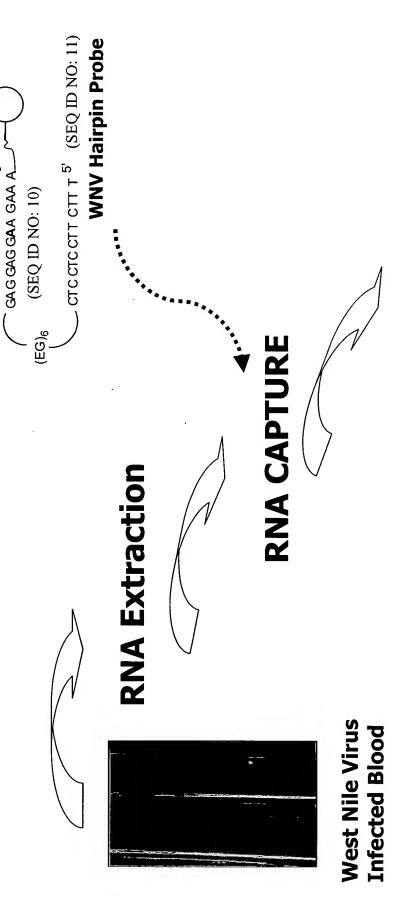
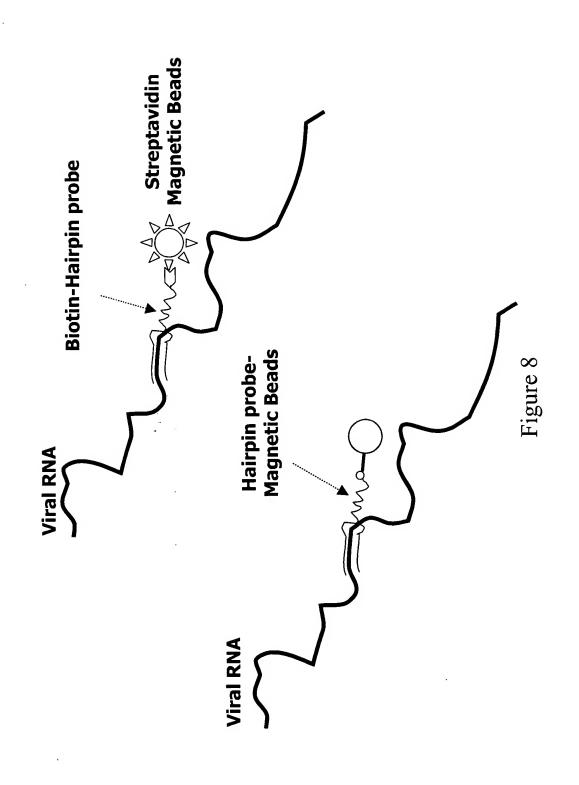
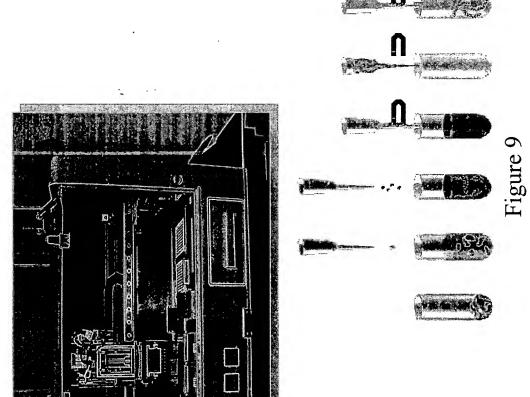


Figure 7

RNA Detection





PYRIMIDINE MOTIF TRIPLEXES

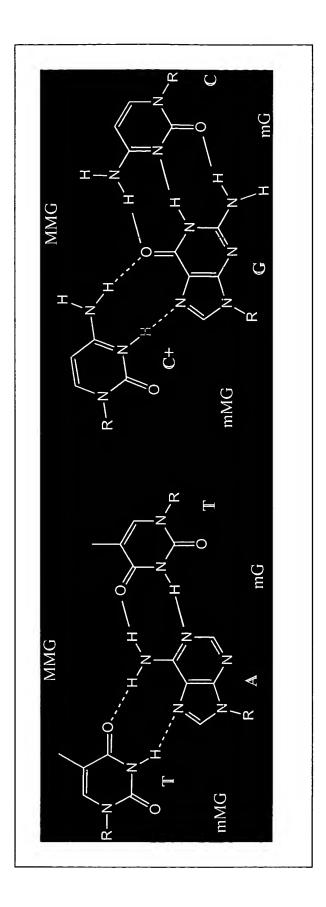
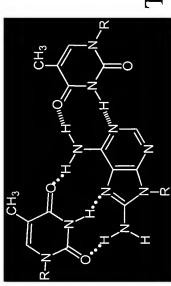


Figure 10

8-aminopurine derivatives



T:8-aminoA:T

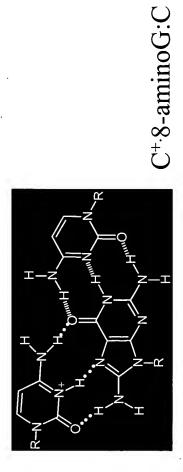
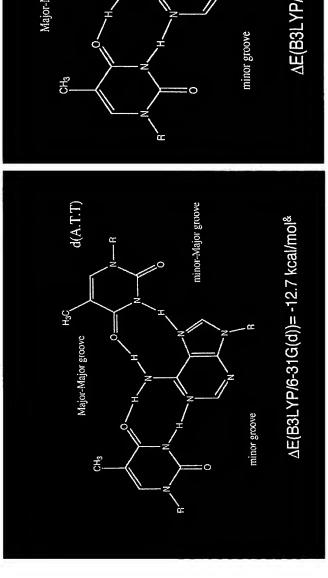
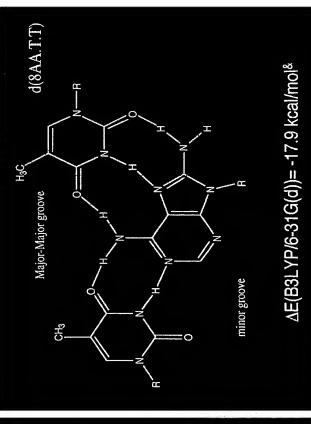


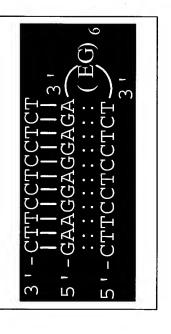
Figure 11





The 8-NH₂ group stabilize 5 kcal/mol the Hoogsteen pair

(SEQ ID NOS: 14, 13, 12)

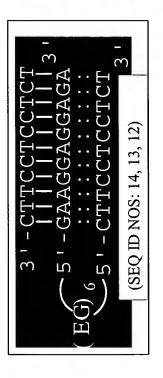


(SEQ ID NO: 15)	G) 6 X= 8-amino-A	(SEQ ID NO: 12)	(SEQ ID NO: 16)	G) 6 Y= 8-amino-G	(SEQ ID NO: 12)
5'-GAAGGXGGXGA	$\sum_{i=1}^{n} (\mathbf{E}_{i}) = \mathbf{E}_{i}$	3 -	5 '-GAAGYAGYAGA) ::::::::	-

Melting temperature (°C) of triplexes [3'-3' hairpins]

Hairpin	5.5 Hq	pH 6.0	pH 6.5	pH 7.0
Unmod.	99	47	36	32
8-aA	62	99	48	46
8-aG	29	59	53	51

Figure 13







Melting temperatures (°C) of triplexes [5'-5' hairpins]

Hairpin	pH 5.5	pH 6.0	pH 6.5	pH 7.0
Unmod.	54	45	33	20
8-aA	57	51	43	34
8-aG	69	59	50	40

Figure 14

Presence of one guanine at the polypyrimidine track

5' TCT CCT GCTTC 3' s₁₁-MMG 5' TCT CCT CCTTC 3' (SEQ ID NO: 17) WC-11mer

(SEQ ID NO: 15) 3 ' AGA^NGGA^NGGAAG

3' TCT CCT **C**CTTC (SEQ ID NO: 12)

(SEQ ID NO: 18)

B-22AMMC

3 ' AGA^NGGA^NCGAAG
3 ' TCT CCT CCTTC
(SEQ ID NO: 12)

Triad 1 Tm (°C)
C.G-C 51

Tm (°C)	47
Triad 1	G.C-C

Figure 15

Melting temperatures (°C) of triplexes containing 2'-O-methyl-RNA



NO: 21)

s'ucuccuccuuc3'

(SEQ ID NO: 22)

Hairpin 11-Me-	l arget 11-Me-RNA
AR22A-RNA 71	—

CD and NMR confirm that parallel duplexes are Hoogsteen (SEQID NO: 15, 12)

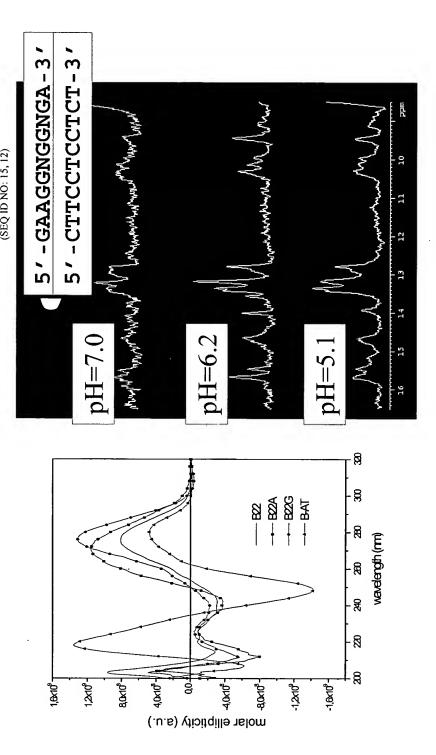


Figure 17

Gel-shift and NMR experiments confirm triplex formation

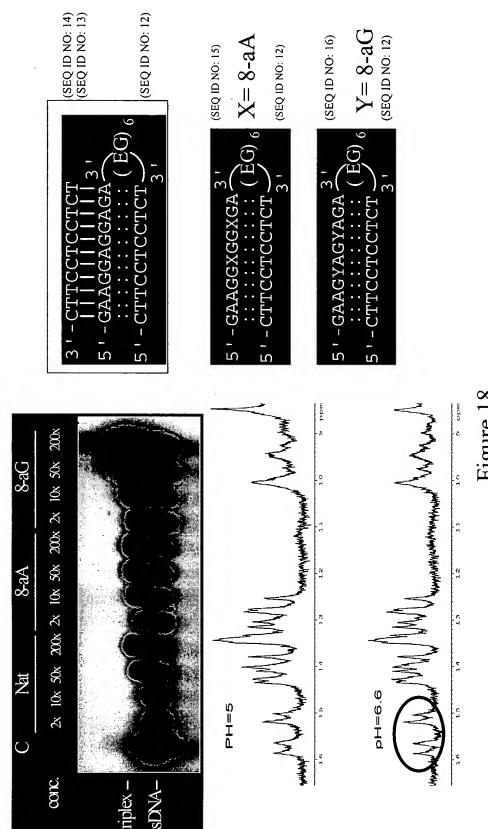


Figure 18



CATESONTCCCOGGI CARANTATARAGGICOATESATOT PATATACACACACAS (AGGISOATAGEATACATAGATATA LITICOCATACOTQIS (NIUCIO DO DIO OCTA ACTACEATOCATAGASACAGASA LA CASA

LOCUS AF404756 11029 bp ss-RNA linear VRL 23-JUL-2002 DEFINITION West Nile virus isolate WN NY 2000-crow3356, complete genome.

VERSION AF404756.1 GI:21929238

ACCESSION AF404756

SOURCE West Nile virus.

Base Position	Sequence Target for Hairpin Design	
2043	СССТТТСТТСА	(SEQ ID NO: 7)
2443	CTCTTCCTCTCCGT	(SEQ ID NO: 2)
3857	СТСТТТСТТСА	(SEQ ID NO: 8)
0299	TTCTTCCTCCTCATGC	(SEQ ID NO: 3)
6740	ссттттстетт	(SEQ ID NO: 9)
6793*	стестстсстт	(SEQ ID NO: 1)
7170	сттссссттсетс	(SEQ ID NO: 6)
7262	CACTCCTTTTTGCC	(SEQ ID NO: 5)
9727	CTCCACTTCCTCAAT	(SEQ ID NO: 4)

Effect of the Hoogsteen strand

	WC-11mer 5' TCT CCT CCTTC 3'	(SEQ ID NO: 15)		3 I TCT CCT CCTTC (SEQ ID NO: 12)	(SEQ ID NO: 15)	3 ' AGA ^N GGA ^N GGAAG B-22Acontrol		3 CCCCTTTTT (SEQ ID NO: 25)	
pH 5.5, 1 M NaCl	Hyperchromicity	+ 12 %	(ss o1 xaldnp)	No transition		+ 22 % (triplex to ss)		+ 12 %	(duplex to ss)
pH 5	Tm (⁰ C)	41		No		57		47	
Target		WC-11mer	(SEQ ID NO: 14)	none		WC- 11mer	(SEQ ID NO: 14)	none	
Hairpin		B-22Acont	(SEQ ID NOS: 15, 25) (SEQ ID NO: 14)	B-22Acont	(SEQ ID NOS: 15, 25)	B-22A	(SEQ ID NOS: 15, 12) (SEQ ID NO: 14)	B-22A	(SEQ ID NOS: 15, 12)